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# EFFECT OF 5-HYDROXYTRYPTAMINE ON PROTEIN SYNTHESIS IN GASTROINTESTINAL AND OTHER TISSUES AND ON SERUM GASTRIN CONCENTRATIONS IN RATS

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**1** The effect of 5-hydroxytryptamine (5-HT) on protein synthesis in the gastrointestinal tissues as well as in the liver, heart and brain was studied by measuring [$^3$H]-leucine incorporation into total tissue protein *in vivo* in rats.

**2** A single injection of 5-HT (10 mg/kg) produced a marked inhibition (45 to 65%) in protein synthesis in the stomach (oxyntic gland area), intestine (jejunum + ileum), colon and brain, but not in the liver and heart.

**3** Dose- and time-dependent studies of 5-HT-induced changes in protein synthesis in the stomach of fed rats revealed that the maximal inhibition of about 65% occurred 1 h after a dose of 12.5 mg/kg.

**4** Animals deprived of food for 24 h differed from their fed counterparts only in the degree of responsiveness, in that a greater inhibition (75%) of [$^3$H]-leucine incorporation into total protein of the stomach was observed 30 min after 5-HT injection.

**5** Pretreatment of animals with methysergide (0.25 mg/kg) essentially abolished the 5-HT-mediated inhibition of protein synthesis in the stomach.

**6** Serum gastrin concentration in fasted animals remained slightly elevated during the initial period of 5-HT treatment (up to 1 h).

**7** The results demonstrate that in certain tissues, 5-HT markedly inhibits protein synthesis. The inhibition in protein synthesis in the gastrointestinal tract cannot be attributed to changes in the concentration of gastrin.

## Introduction

Although the major part of the total body 5-hydroxytryptamine (5-HT) is present in the digestive tract (Erspamer, 1954; 1966), the functional significance of this amine is not fully understood. Administration of 5-HT reduces basal as well as pentagastrin-, histamine- and insulin-stimulated gastric acid secretion (Black, Fischer & Smith, 1958; Håkanson, Lilja, Owman & Thunell, 1967; Hano, Bugajski, Danek & Wantuch, 1975; Jaffe, Kopen & Lazan, 1977). Duodenal acidification, which inhibits gastric acid secretion, is associated with endogenous release of 5-HT (Resnick & Gray, 1962; Jaffe *et al.*, 1977). 5-HT also reduces pepsin secretion in the stomach (Håkanson *et al.*, 1967; Hano *et al.*, 1975). These and other related observations indicate a possible antisecretory role for 5-HT in the stomach.

In an effort to evaluate the changes in cellular functions following 5-HT administration, protein synthesis in gastrointestinal tract (stomach, intestine and colon), liver, heart and brain was examined.

The antral hormone, gastrin, has been established as an important trophic hormone for certain tissues of the gastrointestinal tract (Johnson, 1976). Chronic as well as single injections of pentagastrin have been shown to stimulate protein synthesis in gastric and duodenal mucosa (Johnson, Aures & Yuen, 1969; Majumdar & Goltermann, 1977, 1978). Serum gastrin concentrations were therefore measured to see whether changes in protein synthesis in the digestive tract after 5-HT administration could be related to alterations in the gastrin concentrations.

## Methods

### *Animals and treatment*

Adult Wistar rats of either sex, weighing between 150 and 225 g were either maintained on a commercial

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laboratory diet (*ad libitum*) throughout or fasted for 24 h. All animals had access to water. They were injected intraperitoneally with either 5-hydroxytryptamine in 0.9% w/v NaCl solution (saline) or an equivalent volume of saline. L-[4,5-$^3$H]-leucine (10 μCi/100 g; 46 Ci/mmol, Radiochemical Centre, Amersham) was injected 30 min before the rats were killed. At various intervals after the 5-HT or saline injection the rats were killed by decapitation and blood was obtained through the neck wound. Serum was recovered by centrifugation at 2000 *g* for 10 min. The stomach (oxyntic gland area), a part of the intestine (the combined jejunum and ileum), the colon, brain, heart and liver were quickly dissected out, washed in saline, and were immediately frozen on solid $CO_2$. The tissues and the serum were stored at −20 C until analyzed.

*Protein and radioactivity measurements*

For determination of protein specific activity (ct $min^{-1}$ $mg^{-1}$ protein), each tissue, except the heart, was homogenized in 10% trichloroacetic acid (TCA). The heart was homogenized in water before precipitation of protein with an equal volume of 20% TCA. The rest of the procedure was the same as described earlier (Majumdar, Greenfield & Roigaard-Petersen, 1973). Protein concentrations were determined by the method of Lowry, Rosebrough, Farr & Randall (1951), and the radioactivity was counted in 10 ml of Insta-Gel (Packard Instrument Co., Downers Grove, Ill., USA) in a LKB-Wallac scintillation spectrometer at a 30% efficiency.

*Determination of serum gastrin concentration*

Serum gastrin concentrations were kindly determined by Professor J.F. Rehfeld of this Institute by radioimmunoassay using an antisera (No. 2604) raised against human synthetic gastrin as described elsewhere (Rehfeld, Stadil & Rubin, 1972).

*Statistical analyses*

Experiments were statistically evaluated with Student's *t* test for non-paired values, $P < 0.05$ being taken as the level of significance.

*Drugs*

5-Hydroxytryptamine creatinine sulphate was purchased from the Sigma Chemical Co., St. Louis, Mo., U.S.A. Methysergide, [*N*-1-(hydroxymethyl) propyl-1-methyl-(4)-lysergamide] maleate, was a generous gift from Drs H. Weidmann and H. Friedli of Sandoz A.G., Basel, Switzerland.

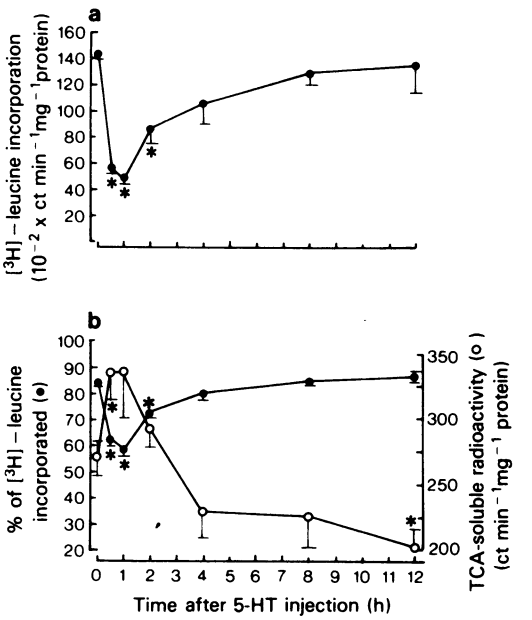


**Figure 1** Time course changes in [$^3$H]-leucine incorporation into total protein of the stomach of fed rats *in vivo* after a single injection of 5-hydroxytryptamine (12.5 mg/kg). In (a) the data for ct $min^{-1}$ $mg^{-1}$ protein are plotted, while in (b) the values for TCA-soluble radioactivity and percentage of [$^3$H]-leucine incorporated are shown. Each value on the curve represents the mean of 6 experiments; vertical lines show s.e. mean. The percentage of [$^3$H]-leucine incorporated was calculated using the formula: [(ct $min^{-1}$ $mg^{-1}$ protein) × (mg protein/g stomach)]/[(ct $min^{-1}$ $g^{-1}$ stomach TCA-soluble fraction) + (ct $min^{-1}$ $mg^{-1}$ protein × mg protein/g stomach)] × 100. * Statistically significant, $P < 0.025$ or lower when compared with the saline-treated control (zero-time). The saline-treated control rats were killed 1 h after the injection.

## Results

*Effects of 5-hydroxytryptamine on protein synthesis in the stomach of fed and 24 h-fasted rats*

In the first series of experiments the time course of changes in protein synthesis in the stomach of fed rats was investigated after a single injection of 5-HT (12.5 mg/kg). The dose was chosen arbitrarily but was similar to the one used by earlier workers for acid secretory studies (Håkanson *et al.*, 1967). Administration of 5-HT produced a prompt reduction in [$^3$H]-leucine incorporation into total protein (Figure 1a). The maximal reduction of 67% was observed 1 h after 5-HT treatment. The values returned essentially to the control level 4 h after 5-HT injection (Figure 1a). On the other hand, the TCA-soluble

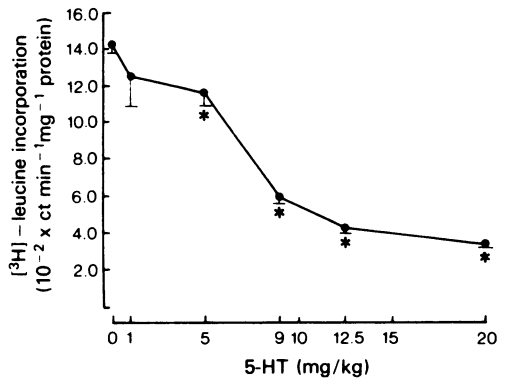


**Figure 2** Effects of increasing doses of 5-hydroxytryptamine (5-HT) on [$^3$H]-leucine incorporation into total protein of the stomach of fed rats *in vivo*. Each value on the curve represents the mean of 6 experiments; vertical lines show s.e. mean. * Statistically significant, $P < 0.025$ or lower when compared with the saline-treated control (zero-dose).

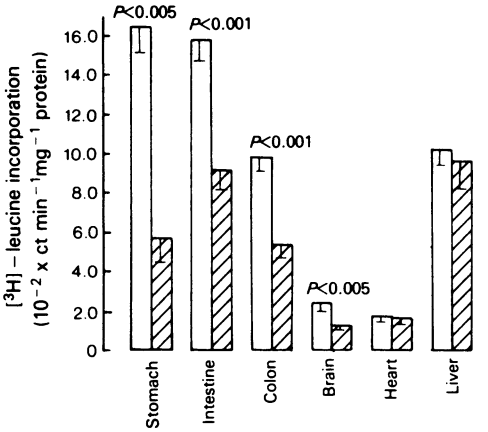


**Figure 3** Effect of a single injection of 5-hydroxytryptamine (10.0 mg/kg) on [$^3$H]-leucine incorporation into various tissues of fed rats *in vivo*; open columns: saline control; hatched columns: 5-HT-treated. Each column represents the mean of 6 experiments; vertical lines show s.e. mean.

radioactivity revealed an inverse relationship with the incorporation data (Figure 1b). A higher TCA-soluble radioactivity (Figure 1b) during the initial period of 5-HT treatment (up to 1 h) suggests that the observed diminution in [$^3$H]-leucine incorporation after 5-HT treatment is not the result of reduced uptake of the precursor amino acid by the stomach. To ascertain further that the inhibition in [$^3$H]-leucine incorporation following 5-HT injection was not associated with changes in the precursor amino acid pool, the results were corrected for TCA-soluble radioactivity, and expressed as a percentage of the total radioactivity incorporated, as described for the correction of RNA specific activity (Majumdar & Jørgensen, 1976). When the results were thus corrected the magnitude of inhibition of [$^3$H]-leucine incorporation by 5-HT over that of the control was reduced (Figure 1b). Nevertheless, the 5-HT-mediated inhibition of protein synthesis was still evident.

In rats fasted for 24 h, 5-HT produced a greater inhibition than in fed rats. Thus inhibition of [$^3$H]-leucine incorporation into protein 30, 60 and 120 min after 5-HT administration was $76 \pm 2.4$, $72 \pm 1.4$ and $51 \pm 4\%$ (mean ± s.e. mean), respectively.

In another experiment on fasted rats, methysergide (0.25 mg/kg i.p.) given 20 min before injection of 5-HT (10 mg/kg) reduced inhibition of [$^3$H]-leucine incorporation from $54 \pm 2.7$ to $19 \pm 6\%$ (mean ± s.e. mean, $n = 6$, $P < 0.1$).

The effect of increasing doses of 5-HT was studied in fed rats, killed 1 h after treatment. A sharp reduction (20 to 60%) in [$^3$H]-leucine incorporation into protein was observed with doses between 5 and 9 mg/kg. Doses above 12.5 mg did not produce any further diminution in amino acid incorporation into total protein of the stomach (Figure 2).

*Effects of 5-hydroxytryptamine on protein synthesis in different tissues of fed rats*

To determine whether the 5-HT-mediated inhibition in protein synthesis is confined to only mucosa or could be observed in other cellular layers of the oxyntic gland area of the stomach, [$^3$H]-leucine incorporation into protein of both mucosa and the rest of the stomach (stomach wall) was measured. The results revealed a similar 65% reduction in amino acid incorporation into total protein of both layers 1 h after the 12.4 mg/kg dose of 5-HT (ct $min^{-1}$ $mg^{-1}$ protein, mucosa = $1480 \pm 26$ (control) and $535 \pm 26$ (5-HT); stomach wall = $1641 \pm 229$ (control) and $527 \pm 57$ (5-HT); $n = 4$). The results indicate that the normal metabolic functions of cells of different layers of the stomach are affected by exogenous 5-HT.

To determine whether 5-HT affects protein synthesis in other organs, [$^3$H]-leucine incorporation into total protein of intestine (jejunum + ileum), colon, brain, heart and liver of the fed rats was studied. The stomach was included for purposes of comparison. The results showed that whereas the amino acid incorporation into protein of stomach, intestine, colon and brains was reduced by 45 to 65% 1 h after the 5-HT injection, protein synthesis in both liver and heart was unaffected by the compound (Figure 3). The maximal inhibition of 65% occurred in the stomach, indicating that the tissue is most susceptible to exogenous 5-HT. The decreased [$^3$H]-leucine incorpor-

ation into protein of stomach, intestine, colon and brain was accompanied by higher levels of TCA-soluble radioactivity (data not shown), suggesting that the lowered protein synthesis is probably not due to decreased amino acid uptake by the tissues.

*Effects of 5-hydroxytryptamine on serum gastrin concentrations in 24 h fasted rats*

Serum gastrin concentrations 0.5, 1 and 2 h after 5-HT (12.5 mg/kg i.p.) were 146 ± 7, 150 ± 7 and 129 ± 13 pg/ml ($n = 6$), respectively. None of these values differed significantly from the control 118 ± 19 ($n = 6$) taken 1 h after injection of saline.

## Discussion

The present experimental results demonstrate that a single injection of 5-HT markedly inhibits [$^3$H]-leucine incorporation into total protein of the stomach, intestine (jejunum + ileum), colon and brain, but not in the liver and heart. The mechanism of the inhibitory action of 5-HT on protein synthesis is, at present, unknown. The amine is known to exert a powerful vascular effect, and in the dog the 5-HT-mediated inhibition of gastric secretion is attributed to decreased blood supply to the gastric mucosa (Rudick, Guntheroth & Nyhus, 1967). On the other hand, 5-HT is found to dilate the gastric mucosal blood vessels in rats (Dolcini, Zaidman & Gray, 1960). Therefore, in the gastrointestinal tissues it seems unlikely that the inhibition of protein synthesis following 5-HT administration could be due to diminished supply of blood-borne factors for protein synthesis. Furthermore, our observation of increased TCA-soluble radioactivity in tissues of the gastrointestinal tract as well as in the brain after 5-HT injection suggests that in none of the tissues could the inhibition of protein synthesis be attributed to reduction in the precursor amino acid supply. The higher TCA-soluble radioactivity during the period of lower protein synthesis indicates a decreased utilization of the precursor amino acid for protein synthesis.

It is not known whether the observed inhibition of protein synthesis in different gastrointestinal tissues as well as in the brain is mediated directly by 5-HT or by some other factor(s). Pretreatment of animals with the 5-HT receptor blocker, methysergide, essentially abolishes the 5-HT-mediated inhibition of protein synthesis in the stomach. Although such an observation suggests a direct action of 5-HT on the stomach protein synthesis, further studies are required to justify this hypothesis. 5-HT did not produce any reduction in the circulating levels of gastrin, a hormone known to exert trophic action on certain tissues of the gastrointestinal tract (Johnson, 1976), indicating that gastrin is not involved in the inhibition of protein synthesis by 5-HT. Although serum gastrin concentrations were slightly elevated after a single injection of 5-HT, this effect was not significant. Such a finding further suggests that the earlier observation of decreased acid and pepsin output following 5-HT administration (Håkanson *et al.*, 1967; Hano *et al.*, 1975; Jaffe *et al.* 1977), cannot be attributed to changes in gastrin concentrations.

The present observation that exogenous 5-HT inhibits protein synthesis in the gastrointestinal tract and brain but not in the liver and heart may indicate a tissue specificity of the compound. However, more detailed experiments on the responsiveness of different tissues to 5-HT are necessary before firm conclusions can be drawn.

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